Summary
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On a global scale, calculating the amount of waste being generated presents a problem. There are number of issues, including a lack of reporting by many countries and inconsistencies in the way countries report. As estimated by OECD, their 25 member countries generated about 4 billion tons of waste in 2001. Natural raw materials and wastes originate mostly from agriculture, food industry and forestry. These are mainly carbohydrates of varying chemical complexity and include sugar, starch, cellulose, hemicellulose and lignin. The waste material is frequently important for economic and environmental reasons. For example many byproducts of the food industry are of low economic value and are often discharged into water ways, creating serious environmental problems. Most of the agricultural by products and wastes are also disposed without proper treatment. Their building up in the environment can present serious pollution problems and therefore their utilization in biotechnological processes albeit at little economic gain, can have overall community value. One of the greatest challenges in biotechnology is the development of suitable technology to enable cellulosic wastes (domestic, industrial, and agricultural) to be used as substrates for fermentation processes.

Since time immemorial, microbial processes have been harnessed for the production of food, pharmaceuticals and alcoholic beverages. In general most raw materials are of naturally occurring substances from which more useful materials can be produced. Recent advances in industrial biotechnology offer potential opportunities for economic utilization of agroindustrial wastes, particularly those originating from tropical regions. In recent years, there has been an increasing trend towards more efficient utilization of agro industrial wastes and residues for the production of bulk chemicals and value added products exploiting microbial metabolism. Application of agroindustrial residues in bioprocesses on the one hand provides alternative
substrates, and on other helps in solving pollution problems, which their disposal may otherwise cause. With the advent of biotechnological innovations, mainly in the area of enzyme and fermentation technology many new avenues have opened for their utilization.

Citric acid is normally produced by surface or submerged fermentation of sucrose or molasses by suitable strains of *A. niger*. Citric acid fermentation is one of the rare examples of industrial fermentation technology where academic discoveries have worked in tandem with industrial know-how, in spite of an apparent lack of collaboration, to give rise to an efficient fermentation process. Because of its commercial and academic importance, the biosynthesis of citric acid by moulds has been a subject of numerous investigations. Citric acid can be produced by different fermentation techniques viz., SmF, LSF and SSF. It is estimated that about 80% of the world production is obtained by submerged fermentation. Since there is great global demand for citric acid, any increase in citric acid productivity would be of potential interest and hence there is an obvious need to consider all possible ways in which this might be achieved. The production by submerged fermentation is still dominating. In the last years, a considerable interest has been shown in using agricultural byproducts and their wastes for production of citric acid by Solid State Fermentation. However, Solid State Processes can create new possibilities for producers. Many byproducts and wastes of the agro industry can be used in the production of citric acid. A cost reduction in citric acid production can be achieved by using less expensive substrates. The use of agroindustrial residues as support in Solid State Fermentation is economically important and minimizes environmental problems. Solid State Fermentation (SSF) processes are of special interest for countries with an abundance of biomass and agro industrial residues. SSF refer to the "cultivation of microorganisms on solid materials in the absence of free liquid". The presence of product in low concentration and the consequent handling, reduction
and disposal of large volume of water during down-stream processing in Submerged Fermentation (SmF) are known as cost intensive, highly problematic and poorly understood unit operations.

SSF offers many advantages over SmF, which include high productivity, less capital investment and relatively easy recovery of the end product. Consequently, a surge of investigations on SSF has been witnessed in the last 20 years throughout the world. In spite of few patents and research papers of exploratory nature on the production of citric acid by SSF, it is not exploited commercially except in Japan and Thailand, mainly due to the lack of data on the optimization of the process and technical as well as economic feasibility.

Keeping the above points in view, the present study was selected to generate basic biochemical and microbiological data on the production of citric acid by SSF process and also to evaluate various agroindustrial wastes that easily available in and around Shimoga district for their potential as solid substrates for citric acid production in SSF system.

Different agro industrial wastes such as Commercial wheat bran (CWB), deoiled rice bran (DRB), areca husk (AH), coffee husk (CH), coconut oil cake (COC), pineapple waste (PAW), sugarcane bagasse (SCB) and cane molasses were procured from different sources and analyzed for their biochemical composition. The analysis indicated that these substrates contained considerable amount of carbohydrates, proteins and other nutrients. Hence these solid substrates were arbitrarily selected for citric acid production by SSF. The solid substrates were also pretreated to ease microbial penetration and metabolism.

A total of 34 strains of A. niger isolated from soil samples collected from various natural resources were screened for their comparative ability to produce citric acid by paper culture technique. Among these, only 14 strains exhibited acid unitage above 2.0. These 14 strains were
further screened for citric acid production in SmF and SSF systems using Millis et al., (1963)
medium and commercial wheat bran medium moistened with mineral salt solution respectively.
Among these, 13 strains were found to produce citric acid in SSF system while all the cultures
were positive for citric acid by SmF. The data indicated that that the strains which produced
higher citric acid in SmF produced lower titres in SSF and vice-versa. This idiosyncracy may be
due to limited water availability in SSF process, high concentration of trace metal ions in CWB
and inability of some strains to tolerate such environment. *A. niger* RCNM 17 highest quantity
of citric acid (23.82g kg⁻¹ dry CWB) in shorter period (72 h) compared to all other strains in SSF
system. Hence *A. niger* RCNM 17 was selected for further study.

A comparative study on citric acid production by *A. niger* RCNM 17 in SmF, LSF and
SSF systems using Shu and Johnson’s medium (1948a) indicated highest citric acid titre
produced in SSF system (61.08g kg⁻¹ dry CWB) in shorter fermentation time (72 h) when
compared to SmF (10.23g l⁻¹ at 168 h) and LSF (24.20g l⁻¹ at 144 h) systems. More over the
sugar conversion rates were very poor in both SmF and LSF. This data established the
superiority of SSF system over other two systems.

Studies were carried out to explore the potential of a number of agro industrial wastes
that are easily available in and around Shimoga district for citric acid production by SSF and to
optimize physicochemical parameters to obtain maximum yield of citric acid. Such data are of
industrial and economic importance in establishing smaller SSF plants throughout the country for
meeting regional demands of citric acid and considerable savings on transportation of raw
materials and products and above all for preventing environmental pollution caused due to
disposal of agroindustrial wastes. Such a step is feasible as SSF plants are economical even at a
lower scale.
CWB generated to a tune of 16-18% contains 13.2% starch on dry weight basis. *A. niger* RCNM 17 produced 30.37±0.72g of citric acid kg⁻¹ dry CWB at 72 h in basal CWB medium moistened with 0.2 M HCl (particle size 0.5-2.5mm, initial pH 4.5 and moisture 50%). When experiments were carried out with different particle size of CWB, the smallest particles (0.5mm) gave better yield. Studies on optimization of pH and initial substrate moisture level indicated improvement in citric acid production and maximum citric acid accumulation at optimum pH 5.0 and moisture level 40% w/w. When trace elements and minerals salts were tested individually for their effect on biosynthesis of citric acid, all of them were found to have positive influence on citric acid production. Any how when they were tested in combination only Fe²⁺, Cu²⁺ and Zn²⁺ were found to have significant effect on citric acid biosynthesis as incorporation of this combination into CWB medium increased citric acid production by 2.16 times when compared to CWB medium with out adding any trace elements.

CWB used in the present study contained 5.68% ash. Still *A. niger* RCNM 17 required additional trace elements to produce higher titres of citric acid. This is in contrary to the strong inhibition of citric acid production by *A. niger* strains in SmF system even at very low levels of trace elements. The present study indicated *A. niger* RCNM 17 has an ability to tolerate high concentrations of trace elements or the trace elements present in CWB may be in bound form or the SSF system was able to overcome hazardous effects of these trace elements.

Among the different carbon sources and nitrogen sources evaluated, sucrose (10% w/w) and ammonium nitrate (0.1%) were found to have positive effect on citric acid production. But the conversions based on the total carbohydrates (initial starch content of CWB plus sucrose supplemented) were not much appreciable. Out of three different lower alcohols (ethanol, methanol and isopropanol) studied, methanol (3%v/w) was found to have profound influence on
citric acid production as it increased citric acid production by 2.23 times when compared to CWB medium without any added methanol. Addition of α- amylase and lipids were found to have no significance. Highest titre of citric acid was obtained with 10%v/w inoculum (1x10^7 spores ml^-1) obtained from 168 h old PDA slant. 10g CWB/ 250 ml Earlenmeyer flask and 60 min autoclaving time were found to be optimal. *A. niger* RCNM 17 produced 106.87±0.82g of citric acid kg^-1 dry CWB under optimized fermentation conditions.

A variety of extra cellular enzymes such as α-amylase, amyloglucosidase, α-galactosidase, pectinase, protease, xylanase and cellulase were produced by *A. niger* RCNM 17 during citric acid production. Data showed that α-amylase titre was highest (5644±5.86 U g^-1 CWB) at 72 h fermentation whereas, the highest titres of amyloglucosidase (5966±7.57 U g^-1 CWB), α-galactosidase (53±2.05 U g^-1 CWB), protease (17880±5.04 U g^-1 CWB), pectinase (34±1.45 U g^-1 CWB), xylanase (14±1.20 U g^-1 CWB) and cellulase (9±0.88 U g^-1 CWB), were recorded at 96 h fermentation.

Extract obtained from the fermented bran was neutralized with calcium hydroxide to precipitate citric acid as calcium citrate and then the filtered moist precipitate was treated with 70% sulphuric acid to liberate free acid. The solution was passed through ion exchange resins (Dowex-50 and Dowex-2) and concentrated in a vacuum evaporator almost to dryness at 35°C. The content was dried at 36 °C to get citric acid monohydrate. The compound so obtained was identified by determining melting point.

The studies on Repeated Batch Solid Substrate Fermentation (RBSSF) with standardized inoculum (10% w/w) in CWB medium under optimal conditions over 10 repeated batches indicated that *A. niger* RCNM 17 produced 104.63±0.47g of citric acid kg^-1 dry CWB at 72 h in
the first RBSSF, followed by 102.57±0.33g and 98.23±0.42g of citric acid kg⁻¹ dry CWB in the second and third RBSSF, respectively. A sharp decline in citric acid production occurred beyond 4th RBSSF.

Paddy when milled leads to the generation of rice bran to an extent of 5-7% of the paddy processed. Rice bran after extraction of oil still contains 20% starch on dry weight basis. *A. niger* RCNM 17 produced 34.68g of citric acid kg⁻¹ dry deoiled rice bran (DRB) in basal DRB medium (initial moisture content 50% w/w and pH 5.0) at 72 h. Incorporation of optimum pH 5.5 and moisture level 35% w/w increased citric acid production by 1.49 and 2.26 times respectively. Addition of standardized trace elements (1 mg Fe²⁺, 0.3 mg Cu²⁺ and 0.2 mg Zn²⁺ kg⁻¹ DRB) resulted in further 45% increase in citric acid production. Supplementation of DRB medium with ammonium nitrate (0.05-0.2% w/w) and sucrose (5-10% w/w) were found to have no significance. Addition of methanol (3% v/w) increased citric acid production by 1.2 times and 3.98 times when compared to DRB medium (initial pH 5.5, moisture level 35% w/w, supplemented with 1 mg Fe²⁺, 0.3 mg Cu²⁺ and 0.2 mg Zn²⁺ kg⁻¹ DRB) and basal DRB medium (initial moisture content 50% w/w and pH 5.0) respectively. 10% v/w inoculum (1x10⁷ spores ml⁻¹) and 30 min autoclaving time were found to be optimal. *A. niger* RCNM 17 produced 139.12 g of citric acid kg⁻¹ dry DRB under optimal conditions.

Areca husk is generated during separation of areca nut from the crop and is usually disposed as waste. Areca husk contains 21% fermentable sugars on dry weight basis. *A. niger* RCNM 17 produced 26.23g of citric acid kg⁻¹ dry areca husk (AH) at 72 h in basal areca husk medium (initial moisture content 50% w/w and pH 3.5). Fermentation under optimal pH 5.0 and moisture level 50% increased citric acid production by 2 times when compared to basal AH medium. Addition of standardized trace elements (1 mg Fe²⁺, 0.3 mg Cu²⁺ and 0.2 mg Zn²⁺ kg⁻¹
AH) shown further increase in citric acid production by 1.32 times. Supplementation of AH medium (initial moisture 50% and pH 5.0, supplemented with 1 mg Fe$^{2+}$, 0.3 mg Cu$^{2+}$ and 0.2 mg Zn$^{2+}$ kg$^{-1}$ AH) with ammonium nitrate (0.05-0.2% w/w) and sucrose (5-20% w/w) found to have no significant effect on citric acid production. Addition of methanol (3% v/w) to AH medium (initial moisture 50% and pH 5.0, supplemented with 1 mg Fe$^{2+}$, 0.3 mg Cu$^{2+}$ and 0.2 mg Zn$^{2+}$ kg$^{-1}$ AH) shown further increase in citric acid production by 1.65 times. 10% v/w inoculum level and 15 min autoclaving time were found to be optimal. *A. niger* RCNM 17 produced 119.86g of citric acid kg$^{-1}$ dry AH under optimal conditions.

Coffee husk (CH), a solid residue generated in the processing of coffee by the dry method contains 24% fermentable sugars on dry weight basis. *A. niger* RCNM 17 produced 82.38g of citric acid kg$^{-1}$ dry CH at 72 h in basal CH medium (initial moisture level 50% and pH 4.0) resulting in 34.32% and 56.09% conversions based on initial sugars and sugars consumed respectively. Incorporation of optimum pH 4.5 and moisture level 45% increased citric acid production by 1.4 and 1.6 times respectively when compared to basal CH medium. Supplementation of standardized trace elements (1 mg Fe$^{2+}$, 0.3 mg Cu$^{2+}$ and 0.2 mg Zn$^{2+}$ kg$^{-1}$ CH) to AH medium (initial pH 4.5 and moisture level 45%) 13.6% increase in citric acid production. Supplementation of either ammonium nitrate (0.05-0.2% w/w) or sucrose (5-20% w/w) found to have no significance. Anyhow addition of methanol (3% v/w) to CH medium (initial pH 4.5 and moisture level 45%, supplemented with 1 mg Fe$^{2+}$, 0.3 mg Cu$^{2+}$ and 0.2 mg Zn$^{2+}$ kg$^{-1}$ CH) showed further improvement in citric acid production 1.28 times. 10% v/w inoculum level and 30 min autoclaving time were found to be optimal. *A. niger* RCNM 17 accumulated 187.13g of citric acid kg$^{-1}$ dry CH at 72 h in under optimal conditions.
Coconut oil cake (COC) is a byproduct obtained after oil extraction and contains 29.5% fermentable sugars on dry weight basis. *A. niger* RCNM 17 produced 74.3g of citric acid kg$^{-1}$ dry COC at 72 h in basal COC medium (initial moisture level 50% and pH 5.0). Citric acid production was increased by 30.1% and 37.7% on incorporation of optimal pH 6.0 and moisture level 60% respectively. Addition of standardized trace elements (1 mg Fe$^{2+}$, 0.3 mg Cu$^{2+}$ and 0.2 mg Zn$^{2+}$ kg$^{-1}$ COC) to COC medium (intial moisture level 60% and pH 6.0) resulted in further 10.5% increase in citric acid production. Supplementation of ammonium nitrate (0.05-0.2% w/w) and sucrose (5-20% w/w) to COC medium (initial moisture level 60% and pH 6.0, supplemented with 1 mg Fe$^{2+}$, 0.3 mg Cu$^{2+}$ and 0.2 mg Zn$^{2+}$ kg$^{-1}$ COC) didn’t show any further improvement. Addition of methanol (3% v/w) to COC medium (initial moisture level 60% and pH 6.0, supplemented with 1 mg Fe$^{2+}$, 0.3 mg Cu$^{2+}$ and 0.2 mg Zn$^{2+}$ kg$^{-1}$ COC) resulted in 37.43% increase in citric acid production. 10% v/w inoculum level and 15 min autoclaving time were found to be optimal. *A. niger* RCNM 17 accumulated 182.79g of citric acid kg$^{-1}$ dry COC at 72 h in under optimal conditions.

A great volume of pineapple waste (PAW) is generated after extraction of juice from pineapple fruit, which normally accounts for about 30% of fruit weight. PAW contains 25.4% fermentable sugars on dry weight basis. *A. niger* RCNM 17 produced 81.26g of citric acid kg$^{-1}$ dry PAW at 72 h in basal PAW medium (initial moisture level 50% and pH 4.8). Incorporation of optimal pH 4.0 and moisture level 65% resulted in 34.5% and 65.2% increase in citric acid production respectively. Supplementation of standardized trace elements (1 mg Fe$^{2+}$, 0.3 mg Cu$^{2+}$ and 0.2 mg Zn$^{2+}$ kg$^{-1}$ PAW) to PAW medium (initial moisture level 65% and pH 4.0) resulted in further 9% increase in citric acid production. Ammonium nitrate (0.05-0.2% w/w) and sucrose (5-20% w/w) supplementation in PAW medium (initial moisture level 65% and pH 4.0,
supplemented with 1 mg Fe$^{2+}$, 0.3 mg Cu$^{2+}$ and 0.2 mg Zn$^{2+}$ kg$^{-1}$ PAW) found to have no significant influence on citric acid production. Anyhow addition of methanol (3% v/w) to PAW medium (initial moisture level 65% and pH 4.0, supplemented with 1 mg Fe$^{2+}$, 0.3 mg Cu$^{2+}$ and 0.2 mg Zn$^{2+}$ kg$^{-1}$ PAW) resulted in further 42.39% increase in citric acid production. 10% v/w inoculum level and 15 min autoclaving time were found to be optimal. *A. niger* RCNM 17 accumulated the highest titre of 208.48 g of citric acid kg$^{-1}$ dry PAW at 72 h.

In a typical sugar factory 100 tons of cane produces 10 tons sugar, 4 tons molasses, 30 tons bagasse and 0.3 tons filter mud. Abundant quantity of bagasse generated after extraction of juice is a fibrous residue that is chiefly composed of cellulose and hemicellulose. Molasses is the residual syrup generated during preparation of sugar by repeated crystallization. Molasses contains 35-40% sucrose and high concentrations of trace metallic ions. *A. niger* RCNM 17 could accumulate only 9.26 g of citric acid at 72 h in sugarcane bagasse (SCB) medium impregnated with cane molasses. Addition of methanol (3% v/w) to the above medium not only increased citric acid production by 5.26 times but also decreased fermentation time by 24 h. Impregnation of SCB with EDTA and potassium ferrocyanide (100 ppm) treated molasses resulted in further 19.90% and 59.90% increase in citric acid production respectively.
Conclusion
CONCLUSION

Citric acid is a versatile and innocuous alimentary additive. It is accepted worldwide as GRAS (generally recognized as safe), approved by the joint FAO/WHO Expert Committee on Food Additives. The food and pharmaceutical industries utilize citric acid extensively because of its general recognition of safety, pleasant acid taste, high water solubility and chelating and buffering properties. Any increase in citric acid productivity would be of potential interest and hence there is an obvious need to consider all possible ways in which this might be achieved. The production by submerged fermentation is still dominating. However, Solid State Fermentation can create new possibilities for producers. Culturing conditions for citric acid production by *A. niger* vary from strain to strain and also depend on the type of process. The optimization of cultural conditions and physicochemical parameters is the key for high and consistent yields of metabolites like citric acid.

The present study indicated that all the agro industrial wastes used have a great potential as solid substrates for citric acid production by *A. niger* RCNM17 in SSF system and also established the superiority of SSF system over traditionally used SmF and LSF systems. *A. niger* RCNM17 could perform very well under Solid Substrate Fermentation conditions and supported maximum production of citric acid under optimal conditions of fermentation. The ability of the fungus to grow on inexpensive solid substrates makes it a potential candidate for commercial exploitation and the well accepted economic advantages of SSF technique might also fulfill the need for reduction in the cost of citric acid. The production of citric acid by SSF making use of inexpensive solid substrates will be of immense value to the fermentation industry. However, further work on scale up of citric acid production with a view to its commercial application is required. The fermented solid substrates after suitable fungicidal treatment or decontamination...
seems to be a promising non-conventional feed alternatives and used for vermicomposting. Besides this selected studies might be conducted in order to develop the present process, which would offer a low-cost technology for using a low-grade and abundant agro industrial wastes. At last, application of tropical agro industrial wastes in bioprocessing by SSF on the one hand may provide alternative substrates, and on other help in solving pollution problems, which their disposal otherwise causes.